DIFFERENTIAL COLORIMETRIC DETERMINATION OF NITRITE AND NITRATE IONS IN WATER

by

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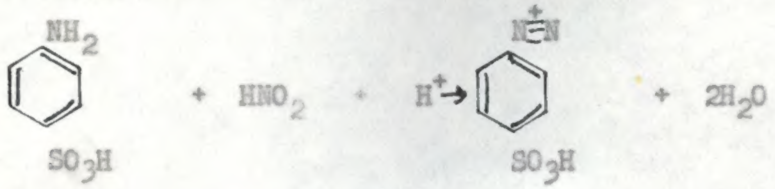
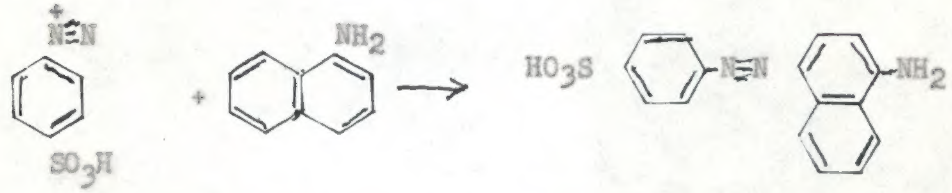
INTRODUCTION

The presence of nitrate and nitrite ions has long been a problem in the purification of water. Removal of either of these constituents by methods other than such costly operations as distillation or ion exchange has not as yet been realized. Consequently, it is common practice in most instances to accept their presence, and on occasions where they exceed a critical limit to dilute or change the source of supply. Due to the toxic nature of these ions, it is necessary to be constantly informed on their concentration.

Nitrate and nitrite ions, as found in water, represent the final oxidation products of nitrogenous organic matter. They are continually being formed and destroyed by organisms as part of the natural nitrogen cycle, and to some extent by direct chemical action. Past history of the water source and climatic conditions are important factors contributing to the quantity of these ions present at any given time. For example, higher concentrations are normally expected after spring flooding when runoff from surrounding terrain is at a maximum, and large amounts of organic matter are being washed into the main river bodies. Of the two constituents, nitrate ion is usually found in greater abundance. This is due to its greater stability and also to the ease with which nitrite ion is oxidized.

Nitrite ion is most frequently determined by the diazo-coupling reaction (1). The dependability of this reaction has few parallels in analytical chemistry, and ever since the classical work of Griess (6) in 1879 it has been the preferred method of analysis. Diazotization of sulfanilic acid followed by coupling of the diazonium cation formed with α-naphthylamine is the recommended standar procedure for nitrite ion determinations in water (1). Colorimetric estimation of the reddish-purple azo dye, α-naphthylamine-p-azobenzene-p-sulfonic acid. produced serves as a means of evalutation. Consideration of the reactions involving the above mentioned amine and coupler aptly illustrate most modifications of diazotization and coupling.

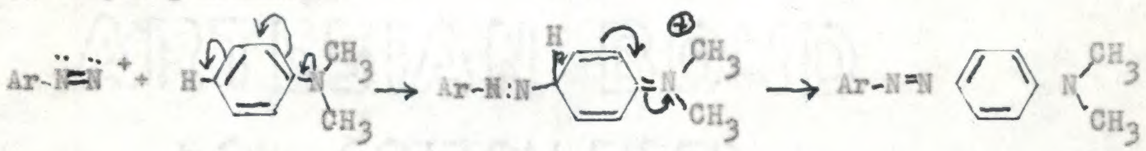




It should be noted that the coupling of α-naphthylamine occurs para to the amino group. A general mechanism explaining this type of occurence on the basis of resonance concepts as they affect the diazonium cation and coupler is given by Saunder (14) in his review of the work of Hauser and Breslow.

$$Ar-\overset{+}{N}\equiv\ddot{N} \rightleftharpoons Ar-\ddot{N}=\ddot{N}^{+} \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} Ar-\overset{H^+}{N}=N-OH \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} Ar-N=N-OH$$

Selecting $Ar-\ddot{N}=\ddot{N}^{+}$ as the active resonance form, they propose the following coupling mechanism:



Numerous combinations of amines and couplers used to form azo dyes appear in the literature, and fairly comprehensive surveys are given by Saunders (14), Venkataraman (16), and Welcher (17).

An interesting modification of the above mentioned reaction is the recent work of Kuemmel and Mellon (7). These workers found that the diazonium cations formed by diazotization of several p-phenylenediamine derivatives had high enough absorption bands in the ultra violet region to be measured directly. The advantage of using such a procedue is the elimination of the coupling step, and the inconvenience of stringent pH control and instability of azo dyes that sometimes accompany it. On the other hand however, the instability of diazonium cations and the need for ultra violet optics to record their absorbance affords a serious limitation.

Nitrate ion has been determined by the use of phenoldisulfonic acid or Nessler's reagent following reduction to ammonia (1). Neither method is ideal, however. The latter requires a rather lengthy procedure, and both are dependent upon a yellow or brown hue for evaluation. The phenoldisulfonic acid procedure is further complicated by the interference of chloride ion. Its removal by the precipitation of silver chloride must be done without the aid of digestion due to the loss of nitrate ion upon heating in the presence of silver salts.

Various colorimetric methods for nitrate ion determinations using such reagents as brucine, xylenol, diphenylamine, and ferrous sulfate have been used with some success. In general they are limited by a lack of selectivity and/or the need for special apparatus. A description of these methods has been included by Snell and Snell (15) in their comprehensive treatise on colorimetric analyses.

A test for nitrate ion using the diazo-coupling reaction was first described by Blom (2). Small amounts of nitrous acid obtained from partial reduction with zinc in acetic acid solution were used to initiate the reaction. While extremely sensitive, the method is not reproducible, and it has been used only for qualitative purposes

A quantitative determination for nitrate ion in soils utilizing the diazo coupling reaction has been reported by Bray (3). By the action of zinc dust in acetic acid solution, in the presence of manganous sulfate, he was able to form reproducible, although nonstoichiometric, amounts of nitrite ion.

Middleton (12) found that 100 per cent recovery of nitrite ion could be effected if the reduction were carried out with zinc in ammoniacal solution in the presence of manganous ion. A critical pH range of 10.2-11.2 at lowered temperatures is a necessary reaction condition. Final evaluation is then based on the orange dye formed by the diazotization and coupling of sulfanilic acid with α-naphthol.

The purpose of the present investigation was to develop a rapid determination of nitrate and nitrite ions in water. Procedures involving separate and mixed determinations of the two ions have been studied. Both are based on the formation of an intense red dye by the diazo-coupling reaction.

Reduction of nitrate to nitrite ion is accomplished with the aid of zinc dust in dilute ammonium hydroxide at 14-15°C., in the presence of manganous hydroxide. (4-aminophenyl)trimethylammonium ion is used as the amine. This is the first time that it has been utilized as an analytical reagent. The coupler, N,N-dimethyl-1-naphthylamine, produces a bright red dye with the diazonium cation derived from the above mentioned amine which is excellent for visual or spectrophotometric comparison.

Two new methods for determining nitrate ion in the presence of nitrite ion and in chlorinated water are described.

For the first case, nitrite ion is destroyed by using it to diazotize the (4-aminophenyl)trimethylammonium ion. The diazonium cation thus formed is removed by exchange onto a nuclear sulfonic cation exchange resin.

Interference with the nitrate ion determination caused by the presence of residual chlorine is overcome by using the chlorine and/or hypochlorous acid to chlorinate the same resin mentioned above. It is unecessary to test for nitrite ion in chlorinated water due to the incompatibility of these substances.

The determinations described cover nitrite and nitrate ions in the ranges of 0-1.5 and 0-2.0 p.p.m. respectively.

Because (4-aminophenyl)trimethylammonium ion is a new analytical reagent, the coupling characteristics of its diazonium cation with twenty-three common biochemical reagents were studied. This is, in part, an extension of the work done by Lambert and Cates (9), who studied the coupling abilities of thirty-three diazonium cations. The results of the present work are to be found in part two of the experimental section of this paper.

A modified determination for nitrate ion using partial reduction techniques is described in part three of the experimental section. This method, while not as sensitive as the one mentioned above, has the advantage of simplicity, and is particularly applicable to field work.

EXPERIMENTAL - PART I

Special Reagents and Equipment

All reagents are of the best available grade.

Standard nitrite ion solution, 1000 p.p.m., 1.500 grams of sodium nitrite per liter of solution.

Standard nitrate ion solution, 1000 p.p.m., 1.631 grams of potassium nitrate per liter of solution.

Amine solution, 1 per cent (4-aminophenyl)trimethylammonium chloride nonohydrochloride. This solution should be stored in a dark bottle.

Coupler solution, 0.5 per cent N,N-dimethyl-1-naphthylamine in 1 per cent hydrochloric acid. This solution should be stored in a dark bottle.

Ammonia solution, 120.0 ml. of concentrated ammonium hydroxide per liter of solution.

Manganese solution, 1 per cent manganous chloride tetrahydrate.

Zinc dust.

Amberlite IR-120 (H) nuclear sulfonic cation exchange resin in the potassium form.

Cadminum iodide-linear starch reagent (8), 11.00 grams of cadmium iodide and 2.50 grams of twice recrystallized linear potato starch fraction per liter of solution.

Magnetic stirrer with 1 5/8 inch polytetrafluoroethylene-covered stirring rod.

Beckman Model DU quartz prism spectrophotometer.

Preparation of (4-aminophenyl)trimethylammonium chloride monohydrochloride. To 43.0 grams of N,N-dimethyl-p-phenylenediamine monohydrochloride dissolved in 650.0 ml. of water at 50°C., add 30.0 ml. of acetic anhydride and stir. Add a solution of 45.0 grams of anhydrous sodium acetate in 150.0 ml. of water. Cool to 20°C. in an ice-water bath. Add a several hundred per cent excess of concentrated ammonium hydroxide to complete precipitation of the product. Collect the precipitate formed by filtration. Wash the precipitate three times on the filter by working it into a slurry with equal portions of a solution containing 75.0 ml. of concentrated ammonium hydroxide and 150.0 ml. of water, and air dry (about 1-2 days). Dissolve the precipitate in 1000 ml. of warm benzene. Filter and add 1000 ml. of benzene at room temperature. Pour the solution into a 2000 ml. standard taper flask and add 32.0 ml. of methyl iodide so that minimum air space remains. Mix thoroughly and stopper tightly without stopcock grease. Store in a dark cool place for four days. Filter, and recrystallize the product from methyl alcohol. Redissolve the product in a minimum amount of hot methyl alcohol. For each gram of product in the preceding step, dissolve 0.7 gram of lead acetate trihydrate in hot methyl alcohol. Mix the two alcoholic solutions, cool, and filter. Bubble hydrogen sulfide gas into the filtrate until precipitation of lead sulfide is complete, and filter. Add 0.6 gram of concentrated hydrochloric acid to the filtrate for each gram of product collected in the recrystallization from methyl alcohol above. Concentrate to about

100-300 ml. (or to where crystals form) with force evaporation on a steam bath. Cool and add just enough methyl alcohol to dissolve all the crystals. Triple the total volume by dilution with ether. After one-half hour or more filter the product. Dissolve in a minimum quantity of methyl alcohol and repeat the ether dilution. Collect the final product by filtration and dry in air. If the product is not colorless, dissolve it in methyl alcohol and decolorize with carbon. Precipitate with ether as previously mentioned and dry.
Yield: Approximately 17 grams.

Preparation of the cation exchange resin. Place the desired amount of Amberlite IR-120 (H) nuclear sulfonic cation exchange resin on a filter. Wash repeatedly with 2 per cent potassium hydroxide until the wash solution remains blue to litmus. Rinse with distilled water until the wash solution is neutral to litmus. Dry in air until the resin beads no longer stick together.

Nitrite and Nitrate Ion Determinations.

Nitrite calibration curve. Prepare nitrite ion samples in the range of 0.1-1.5 p.p.m. To 30.0 ml. of the samples add 1.00 ml. of the amine solution and stir well. Add 1.00 ml. of the coupler solution and acidify with five or six drops of concentrated hydrochloric acid. Dilute to exactly 50.0 ml. After twenty minutes record absorbance readings with a spectrophotometer at 522 mμ.

Nitrate calibration curve. Prepare nitrate ion samples in the range of 0.1-2.0 p.p.m. To 30.0 ml. of the samples, in a 125 ml.

Erlenmeyer flask, add 5.00 ml. of the ammonia reagent and 1.00 ml. of the manganese reagent. Place the flask in a large crystallizing dish containing water at 14-15°C., which is mounted on a magnetic stirrer. (Two thin asbestos mats should be placed on the surface of the stirrer to serve as insulation against heat. At normal room temperatures this bath will remain in the critical temperature range for sufficient time to make a single determination.) Add 0.2 gram of zinc dust and stopper tightly with an unlubricated glass stopper. Stir for six minutes at a constant rate. Filter the solution with suction through a sintered glass filter. After the filter has been sucked dry, rinse the flask with two small portions of distilled water and run them through the filter. Wash the filter with one small portion of distilled water. Add 1.00 ml. of the amine solution to the clear filtrate and stir well. Acidify dropwise with 1.2 ml. (about 15 drops) of concentrated hydrochloric acid and stir well. Add 1.00 ml. of the coupler solution and stir well. After twenty minutes dilute to exactly 5 .0 ml. and record absorbance readings on a spectrophotometer at 522 mμ.

If a constant temperature bath is available, it may be convenient to circulate water between the bath and the crystallizing dish.

Quantitative formation of nitrite ion is critically dependent upon the time and rate of stirring. It is essential that these variables be determined on standard samples prior to attempting an unknown analysis. The stirring rate may be conveniently regulated by adjusting the stirrer just below that point at which splashing occurs. The time can then be varied by fixed increments below and above the six minutes recommended until the correct period is determined.

The amount of zinc dust used is not critical. Once a general idea of the volume associated with 0.2 gram has been established, subsequent portions may be estimated with a spatula.

Test for nitrate ion in the presence of nitrite ion. Test the unknown solution for nitrite ion by placing about one ml. on a spot plate and adding several drops of the amine and coupler reagents along with a drop of concentrated hydrochloric acid. A red color indicates the presence of nitrite ion. If present, remove a 50.0 ml. sample, add 1.00 ml. of the amine and a drop of concentrated hydrochloric acid. Stir with a magnetic stirrer for four minutes. Add five grams of the cation exchange resin and continue stirring for another four minutes. (This amount of resin may be more conveniently measured as one level standard teaspoonful). After the resin has completely settled, test one ml. of the clear liquid for nitrite ion. If the test is positive continue stirring until no red color is obtained. Carefully decant the solution from the resin. Run a nitrate ion determination on a 30.0 ml. sample as described above.

This procedure was successfully used on nitrate ion samples containing 5.0 p.p.m. nitrite ion or less.

Test for nitrate ion in chlorinated water. Test the unknown solution for chlorine or hypochlorous acid by adding a drop of cadmium iodide-linear starch reagent to about 1.0 ml. of the sample on a spot plate. A blue color indicates the presence of chlorine or hypochlorous acid. If present, remove a 100 ml. sample and add 20 grams of the cation exchange resin. Stir for twenty minutes on a magnetic stirrer. Test one ml. again for chlorine or hypochlorous acid. If the test is positive continue stirring until no blue color is obtained. Decant the solution from the resin. Run a nitrate ion determination on a 30.0 ml. sample as described above.

This procedure was successfully used on samples prepared by making standard additions of nitrate ion to chlorinated drinking water.

Dicussion of Complete Nitrite Ion Recovery Method

A mechanism for the formation of nitrite ion by reduction with zinc and manganous hydroxide in ammoniacal solution is not immediately obvious. Experiments conducted by the author and Dr. Jack L. Lambert, his research director, indicate that somewhat less than 70 per cent nitrite ion recovery is realized when the reduction is performed in the absence of manganous hydroxide. Gray (4), who used manganous sulfate as a source of manganous hydroxide, suggested that its purpose is to adsorb chloride ions which might otherwise interfere. Mellor (11), however, in a review of the work done by Patton, shows that manganous hydroxide will not adsorb chloride ions in the presence of sulfate. This, plus the fact that manganous chloride was used in the present procedure, indicates that chloride ion is not an interference and that the role of manganous hydroxide remains uncertain.

Presumably, any nitrite ion not recovered in the absence of manganous hydroxide is further reduced to lower oxidation states of nitrogen, and possibly ammonia. For this reason, it might be proposed that the function of the manganous hydroxide is to form a nitrite complex which is stable to further reduction at the temperature used.

The ability of manganous hydroxide to act as an oxygen scavenger is well known. With respect to the present discussion, however, its purpose is this capacity is not apparent.

Equations depicting the overall nitrate ion reduction and dye formation proceed accordingly:

$$NO_3^- + Zn + 4\,NH_3 + H_2O \longrightarrow NO_2^- + Zn(NH_3)_4^{++} + 2\,OH^-$$

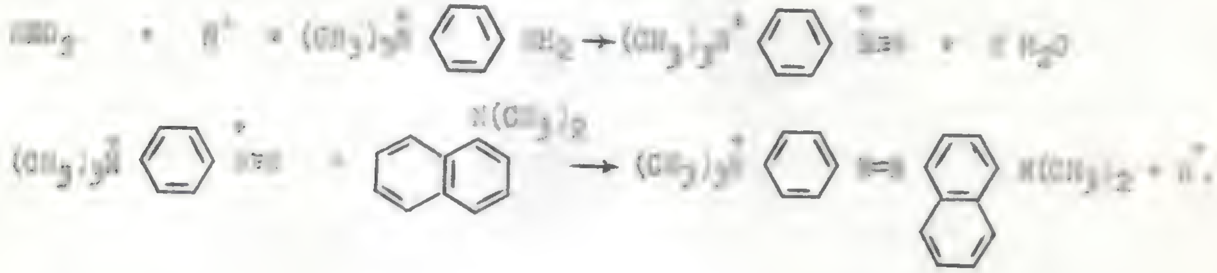


Plate I illustrates the relationship between the nitrite and nitrate ion calibration curves. It is apparent from the diagram that quantitative recovery of nitrite ion is obtained. This is based on the premise that 1.00 gram of nitrate ion is stoichiometrically capable of producing 0.74 gram of nitrite ion. Each point on the curves is an average of five trials.

Plate II indicates the absorption maxima of the dye formed from a 1.0 p.p.m. nitrate ion solution. The broad plateau extending from about 518-530 mμ suggests its applicability to spectrophotometric determinations.

Table I lists the results of standard additions of nitrate ion to natural and treated water samples. The values shown represent and average of five determinations for each concentration indicated. Before addition, the samples contained from about 0.8-3.0 p.p.m. nitrate ion.

EXPLANATION OF PLATE I

Relationship between the nitrite and nitrate ion calibration curves.

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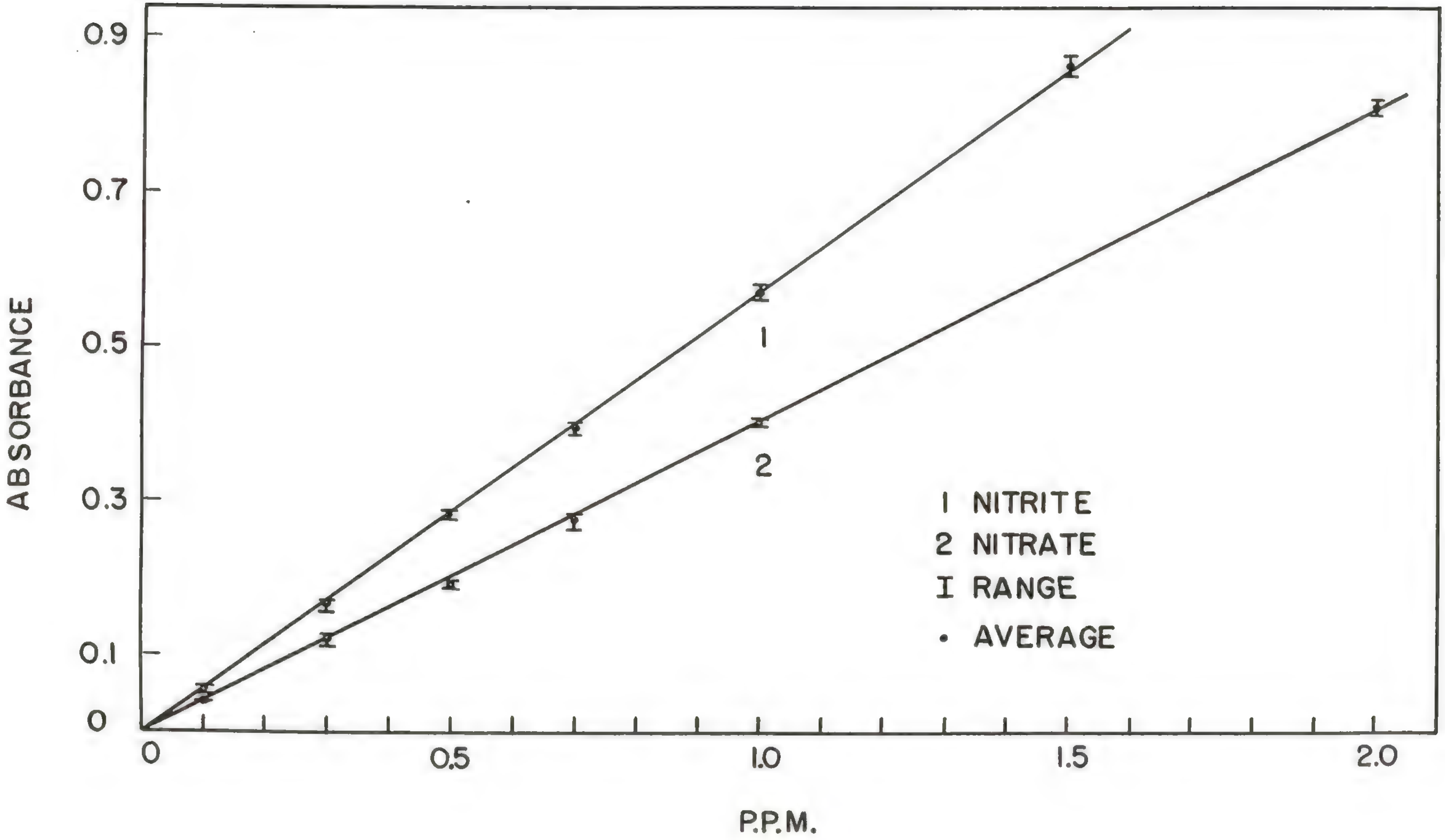

0.9
0.7
0.5
0.3
0.1
0
ABSORBANCE
0
0.5
1.0
1.5
2.0
P.P.M.
1
2
1 NITRITE
2 NITRATE
I RANGE
• AVERAGE

EXPLANATION OF PLATE II

Absorption curve in the visible region of the red azo dye formed by the diazotization and coupling of (4-aminophenyl)trimethylammonium ion with N,N-dimethyl-1-naphthylamine.

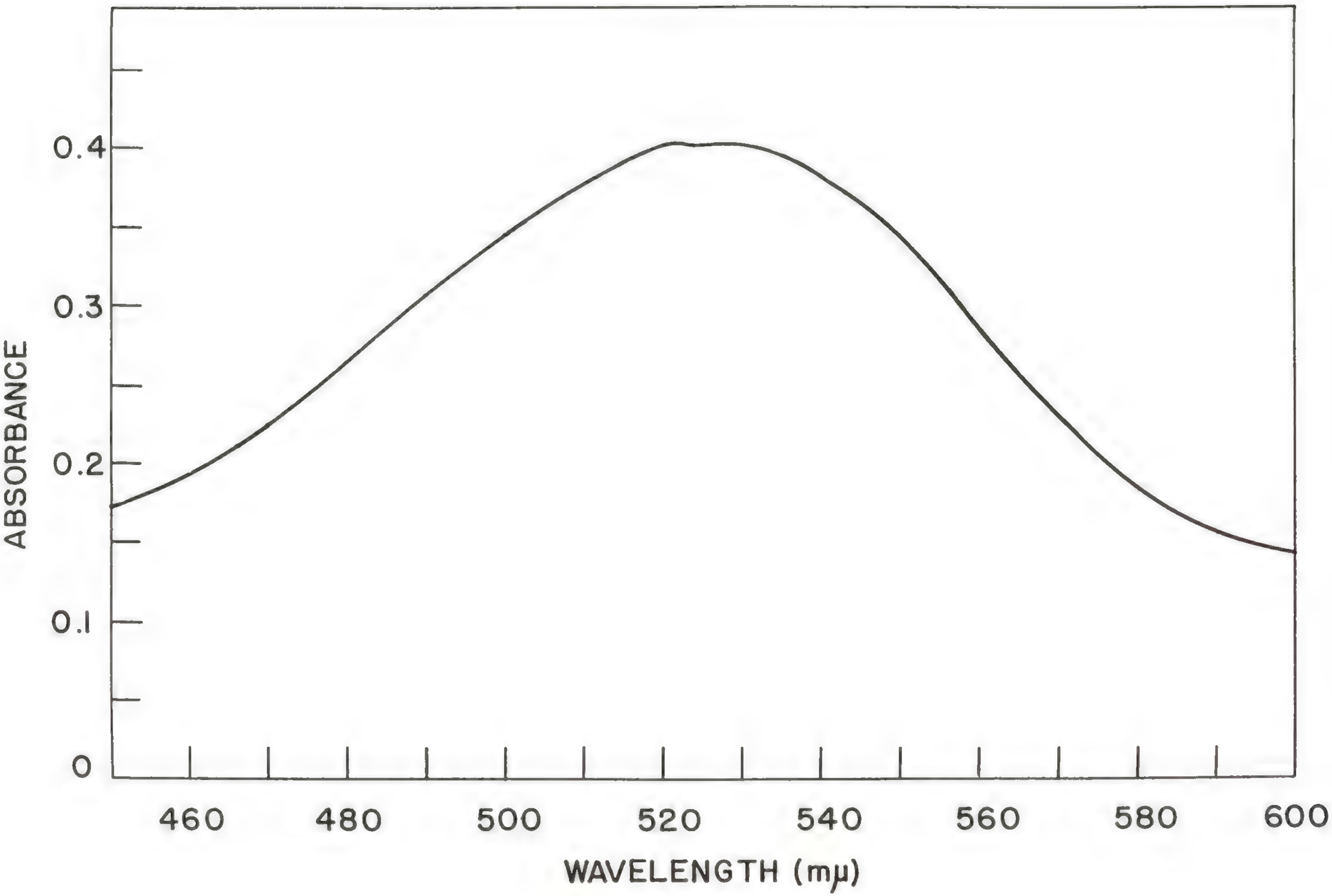

ABSORBANCE
0.4
0.3
0.2
0.1
0
460
480
500
520
540
560
580
600
WAVELENGTH (mμ)

Table I. Analysis of typical water samples.

Source of sample	NO_3^- added p.p.m.[3]	NO_3^- recovered p.p.m.[3]	Deviation in p.p.m.
A[1]	0.10	0.11	0.06
	0.30	0.33	0.04
	0.50	0.55	0.05
	1.00	1.00	0.03
B[2]	0.10	0.12	0.04
	0.30	0.32	0.04
	0.50	0.48	0.02
	1.00	0.98	0.01

[A] Blue River water collected near Manhattan, Kansas, with suspended solids removed by filtration and/or centrifugation.

[B] Manhattan, Kansas, city water supply.

[1] Nitrite ion removed by procedure described here.

[2] Dechlorination accomplished by procedure described here.

[3] Each value represents the average of five trials.

Aliquot portions were taken when the nitrate ion concentration exceeded the limit of the calibration curve.

It is to be noted that the deviation of the standard additions is generally higher than those on the calibration curve. This is attributed to the concentration factors involved. Obviously a 0.1 p.p.m. addition to samples already containing 3.0 p.p.m. will deviate more than a comparison of solutions made up to contain 0.1 p.p.m.

The nitrate ion reduction takes place in a solution having a pH of about 10.8. This falls into the range of 10.2-11.2 described by Middleton (12).

Selection of (4-aminophenyl)trimethylammonium ion was based on its speed of diazotization, coupling ability, and stability in aqueous solution. Pinnow and Koch (13) described a simpler method of preparation for this amine, but the product described here is more suitable for analytical work. Solutions stored for more than six months in a dark bottle were still usable, as were those prepared fresh from the hydrochloride salt which had been synthesized two years earlier.

If a slight yellow hue forms in the amine solution on standing, it can be removed with carbon at room temperature. Carbon thus used may introduce a trace of nitrate ion, but this is of no significance to either the nitrate or nitrite ion determinations. Nitrate ion will not interfere with the nitrite ion determination, and since the amine solution is added after the zinc reduction step when testing for nitrate ion, any additional contaminant will go undetected. A blank determination would be necessary when running a mixed analysis of the two ions, however, since then the amine is present during the nitrate ion reduction.

The coupler was chosen for its ability to couple in acid solution and formation of a favorable color. It has been previously used for colorimetric determinations in alcoholic solutions (10), (15), in which it is reported to be stable.

Low results were obtained in this procedure, however, when ethanol was used as a solvent. This presumably was due to competing reactions between ethanol and the diazonium cation to form an ether and the normal coupling product.

The aqueous hydrochloride salt solution recommended here has a tendency to undergo decomposition and is best prepared daily. Since clarity of solution is not an indication that no decomposition has occured, the condition of the coupler should be checked with a standard solution before running an unknown analysis.

Decomposition, probably due to air oxidation, may affect the pure N,N-dimethyl-1-naphthylamine once the original bottle has been opened. Storage over granular, unamalgamated zinc apparently minimizes this decomposition.

It is interesting to note that when the coupler is prepared in alcoholic solution, only the nitrate ion readings are lowered. The decrease in dye formation being about 10 per cent less than that obtained with the aqueous hydrochloride salt. Absorbance readings taken on nitrite ion determinations show good agreement regardless of which solvent is used. This might suggest some possible influence of either zinc, manganese or ammonium ions on the reaction of the diazonium cation with ethanol mentioned above.

The red hue of the azo dye appears to be stable. Absorbance readings taken on solutions up to one week after color development do not vary significantly. A series of couplers, including the commonly used N-(1-naphthyl)-ethylenediamine, do not yield as intense a color at a given nitrite or nitrate ion concentration when substituted for N,N-dimethyl-1-naphthylamine in this procedure.

When removing nitrite ion, it may be necessary to permit a longer time for completion of diazotization than the four minutes recommended before introducing the cation exchange resin. This period depends on the nitrite ion concentration, and should be determined on standard solutions before attempting an analysis.

Dechlorination of the treated water samples is apparently accomplished by direct chlorination and possibly some addition across any unsaturation in the resin. The possibility of exchange taking place with positive chlorine is considered to be remote.

The following general equations relating concentration to absorbance were derived from the calibration curves. Equation 1 applies to nitrite ion and equation 2 to nitrate ion:

$$(1) \quad C_{NO_2^-} = 1.79 A$$

$$(2) \quad C_{NO_3^-} = 2.44 A$$

where C is concentration in p.p.m., and A is absorbance. The molar absorban . index for the dye formed is 26,200.

EXPERIMENTAL PART II

Some Coupling Characteristics of (4-trimethylammoniumphenyl)-diazonium cation

To further study the coupling behavior of the diazonium cation formed by diazotization of (4-aminophenyl)trimethyl ammonium ion, the procedures of Lambert and Cates (5, 9) were used to effect its transfer to a cation exchange resin. The cation thus isolated can be safely stored, and easily discharged from the resin by the action of excess potassium chloride, thereby becoming readily available for immediate use.

The colors observed from the coupling of the diazonium cation with twenty-three common biochemical reagents in different pH ranges are listed in Table II.

Of the compounds tested, the dye formed from phenol was selected for further quantitative investigation. Its linear relationship in accordance with Beer's Law is apparent from consideration of the curves in Plate III.

It is to be noted that the pH values of the solutions tested are lower than those expected from the buffer solutions used. This is attributed to some hydrogen ion introduction from the resin on which the diazonium cation was dispersed.

Special Reagents and Equipment

All reagents are of the best available grade.

(4-trimethylammoniumphenyl)diazonium cation suspended on Amberlite IR-120 (H) synthetic cation exchange resin, (prepared by the method of Lambert and Cates (5, 9).

Table II. Colors of the azo dyes formed by the coupling of 4-[illegible]diazonium cation with various biochemical compounds.

Compound	pH of solution				
	Unbuffered solution	4.3	6.4	7.8	12.5
l-Tyrosine	col	col	col	o	lt.r
dl-Histidine	col	col	ft.y	y-lt.o	y
dl-Tryptophane	col	ft.y	ft.y	lt.y	lt.y
Pyridoxine hydrochloride	col	ft.y	y-o	y-o	o
Thiamine hydrochloride	col	col	col	ft.o	lt.r
Riboflavin	n.c.	n.c.	n.c.	n.c.	n.c.
Nicotinic acid	col	col	col	col	col
Bilirubin	n.c.	n.c.	n.c.	n.c.	n.c.
Indoleacetic acid	col	g-y	g-y	ft.y	y
Calcium pantothenate	col	col	col	col	col
Folic acid	n.c.	n.c.	n.c.	n.c.	n.c.
Ascorbic acid	ft.y	col	col	col	y
Choline chloride	col	ft.y	col	col	lt.y
i-Inositol	col	col	col	ft.y	ft.y
Bacto-oxgall	n.c.	n.c.	n.c.	lt.y	lt.y
Cholesterol	col	col	col	ft.y	lt.y
Phloroglucinol	y-o	o	o	o	o
Resorcinol	lt.y	y-o	y-o	o	lt.b
Phenol	col	ft.y	y	y	y
Acetonylacetone	col	col	lt.y	y	y
Histamine dihydrochloride	col	col	ft.y	o	o
Imidazole	col	ft.y	ft.y	y-o	g-y
o-Cresol	col	g-y	g-y	o	y-o
Water	col	col	col	col	col

Legend of colors.

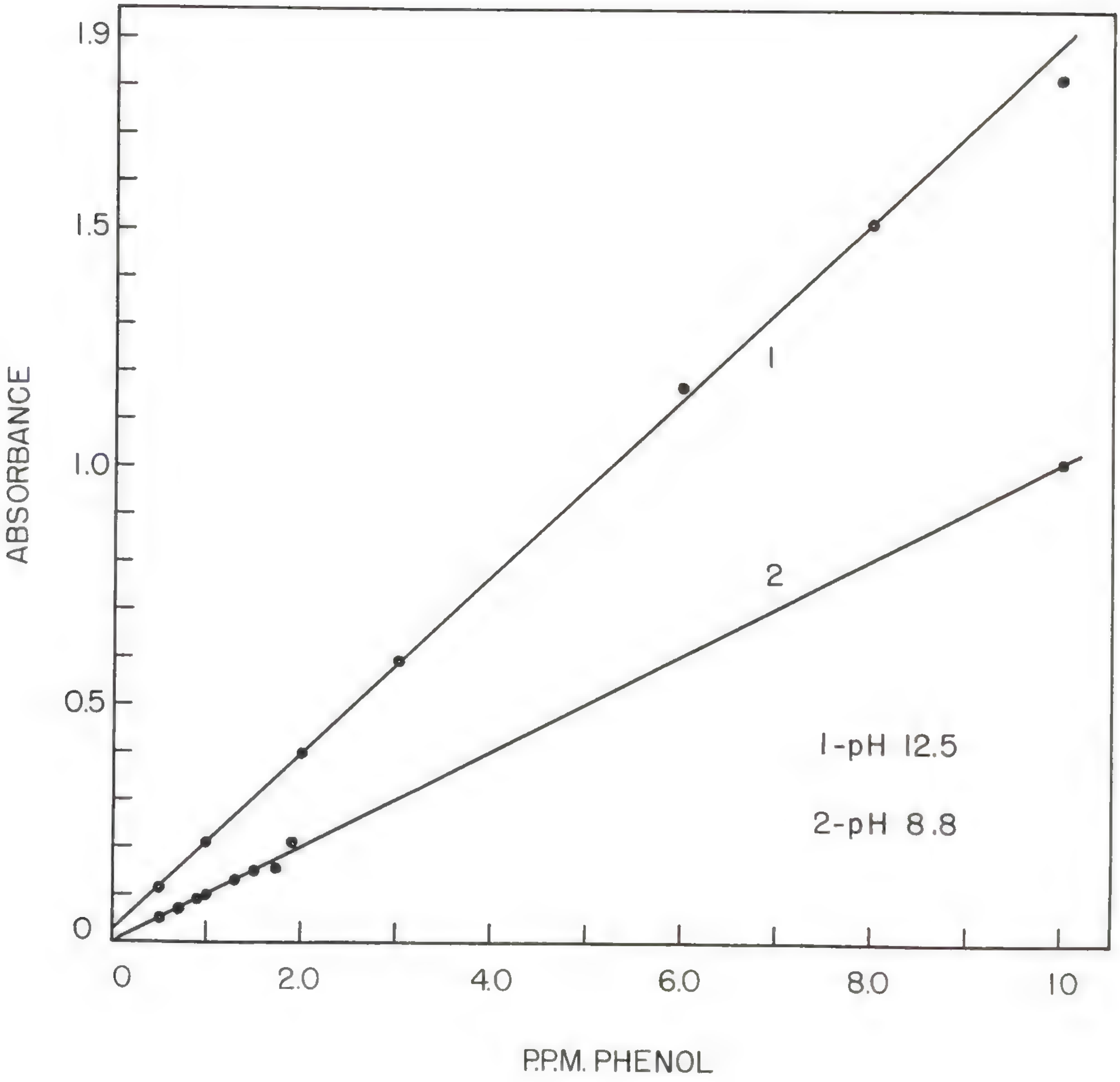
col.,	colorless	b,	brown	r,	red
ft.,	faint	g,	green	y,	yellow
lt.,	light	o,	orange		

n.c., no change (where the solution had some color itself)

EXPLANATION OF PLATE III

Absorption curves of the dye formed from phenol at pH 8.6 and 12.5, (recorded at 445 mμ.)

[illegible] III


ABSORBANCE
0
0.5
1.0
1.5
1.9
0
2.0
4.0
6.0
8.0
10
P.P.M. PHENOL
1
2
1-pH 12.5
2-pH 8.8

l-tyrosine.

dl-Histidine.

dl-Tryptophane.

Pyridoxine hydrochloride.

Thiamine hydrochloride.

Riboflavin.

Nicotinic acid.

Bilirubin.

Oxalacetic acid.

Calcium pantothenate.

Folic acid.

Ascorbic acid.

Choline chloride.

i-Inositol.

Bacto-oxgall.

Cholesterol.

Phloroglucinol.

Resorcinol.

Phenol.

Acetonylacetone.

Histamine dihydrochloride.

Imidazole.

o-cresol.

Beckman Model DU quartz prism spectrophotometer.

Standard spoon measures.

Buffer Solutions

pH 5, 1 M sodium acetate and 1 M acetic acid. Use 68.0 grams of sodium acetate trihydrate and 28.6 ml. of glacial acetic acid per 500 ml. of solution.

pH 7, 2 M ammonium acetate. Use 77.1 grams per 500 ml. of solution.

pH 9, 2 M dipotassium hydrogen phosphate. Use 228 grams of dipotassium hydrogen phosphate trihydrate per 500 ml. of solution.

pH 12, 1 M sodium carbonate. Use 106 grams of sodium carbonate, anhydrous, per 500 ml. of solution.

Qualitative investigations

0.1 per cent solutions of the compounds to be tested were prepared. (Where solubility factors precluded this, saturated solutions were used instead). To one ml. of these solutions, five ml. of the buffer reagents or water were added with stirring, and then one ml. of the (4-trimethylammoniumphenyl)diazonium cation solution was added. Colors were judged after several minutes standing.

The diazonium cation solution was prepared by mixing one-fourth teaspoon (level measure) of potassium chloride and one level teaspoon of the diazonium ion-resin combination with 50 ml. of water.

Quantitative Investigations

Quantitative determinations were made by preparing solutions of phenol in the range of 0-10.0 p.p.m. Ten ml. of these solutions were pipetted into a 50 ml. beaker along with 2.0 ml. of the buffer solution and 1.0 ml. of the diazonium ion solution. After thirty minutes absorbance readings were recorded at 445 mμ.

EXPERIMENTAL PART III

Modified Nitrate Ion determination Using Partial Reduction

In the course of the present investigation, it was found that a simplified modification of the nitrate ion determination described earlier could be used. The basis of this modification entails the elimination of the manganous hydroxide addition as well as the critical control of temperature. The same amine and coupler are used, with the exception that the coupler is prepared in alcoholic solution, which is stable for at least 60 days (15).

The modified procedure does not afford stoichiometric reduction of nitrate to nitrite ion. However, the results obtained are sufficiently reproducible to suggest its use as an acceptable quantitative method.

Special Reagents and Equipment

Unless otherwise indicated, the same reagents are used as in the previous nitrate ion procedure.

Coupler solution, 1.0 ml. of N,N-dimethyl-1-naphthylamine per 250 ml. of 95 per cent ethyl alcohol. This solution should be stored in a dark bottle.

Zinc metal, unamalgamated, granular, 20 mesh.

Standard spoon measures.

Nitrate Calibration Curve

Prepare nitrate ion samples in the range of 0.1-5.0 p.p.m. To 30.0 ml. of the samples in a 125 ml. Erlenmeyer flask, add 5.0 ml. of the ammonia reagent and one-half teaspoonful of zinc. Stir for three and one-half minutes at a constant rate. Decant the supernatant liquid, rinse the flask with two small portions of water, and add the wash solution to the decanted liquid. Add 1.00 ml. each of the amine and coupler solutions. Acidify dropwise with concentrated hydrochloric acid until the clear red color of the dye appears, then add five or six drops in excess. Dilute the red solution to exactly 50.0 ml. and record absorbance readings at 522 mμ on a spectrophotometer after 20 minutes.

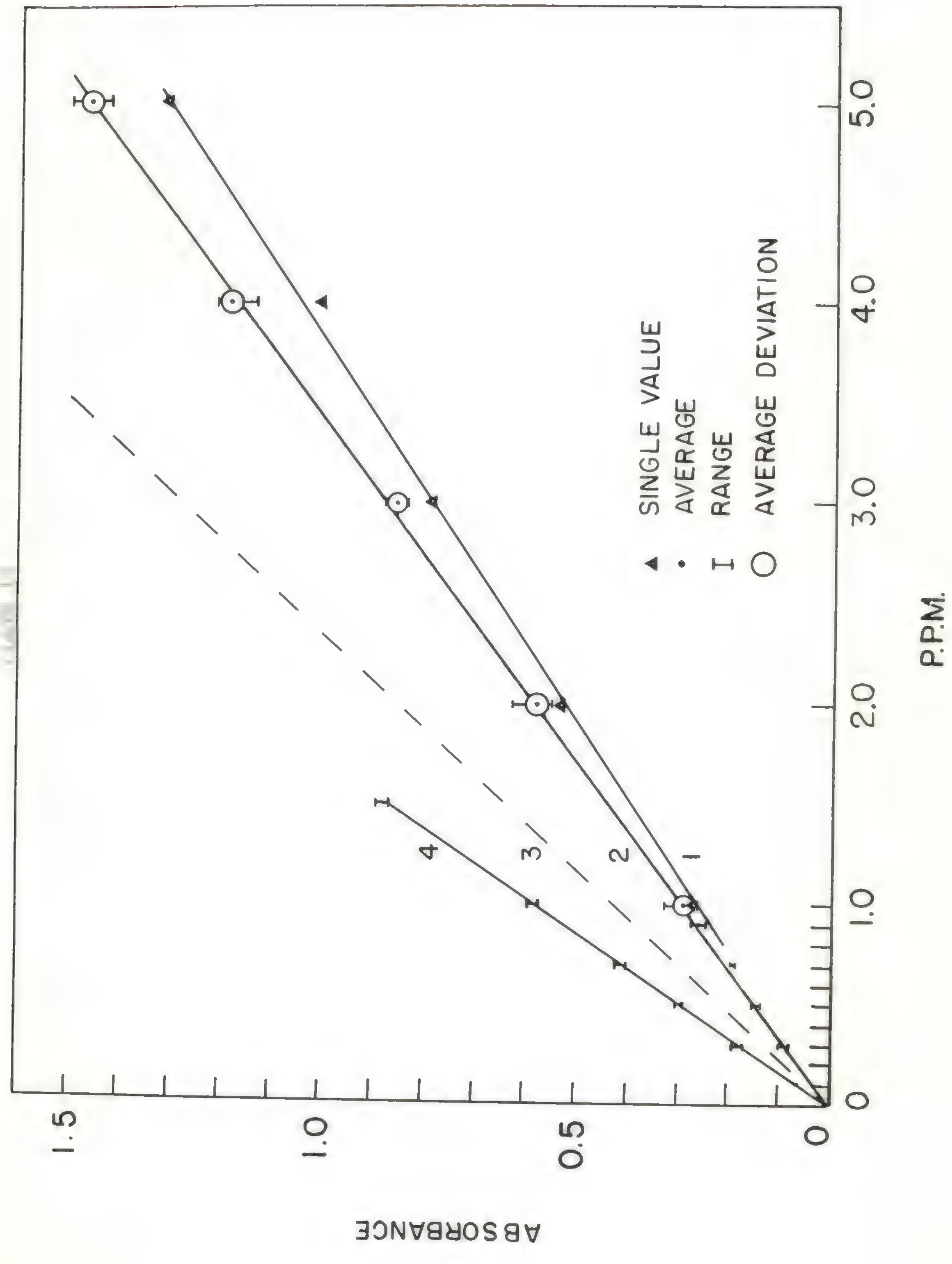
It is essential that the time and rate of stirring be the same for each trial. The combination which produces the most intense color should be determined on standard samples. For the time listed above, a magnetic stirrer operating at approximately 600 cycles per minute (by stroboscopic measurement) was used.

The stirring rate can be conveniently regulated by adjusting the stirrer just below the splashing point. A certain minimum speed is needed to overcome friction due to the rough layer of granular zinc. The range of difference between these extreme rates is sufficiently small to effect a fairly constant value. Values obtained for five successive runs are plotted in Plate IV, curve 2.

When stirring was done by hand (at a rate established by the oper-

EXPLANATION OF PLATE IV

Relationship of the modified nitrate ion calibration curve (curve 2) to the theoretical nitrate ion curve (curve 3) and the nitrite ion calibration curve (curve 4). Curve 1 indicates the results of a single series of nitrate ion determinations using the hand stirring method.


ABSORBANCE
1.5
1.0
0.5
0
P.P.M.
0
1.0
2.0
3.0
4.0
5.0
1
2
3
4
▲ SINGLE VALUE
• AVERAGE
I RANGE
○ AVERAGE DEVIATION

ator), a rapid swirling motion for four minutes and one teaspoonful of zinc were employed. A vigorous shaking motion is undesireable due to the possibility of releasing fine zinc particles into suspension, which would interfere with the final color. The results of a single series of trials using the hand stirring method are shown in Plate IV, curve 1.

Discussion of Modified Nitrate Ion Determination

Success of this determination rests with the ability to form reproducible amounts of nitrite ion by maintaining a constant time and rate of stirring for all samples. The concentration-absorbance relationship under these conditions is linear in accordance with Beer's Law.

Plate IV illustrates the relationship between the experimental nitrate ion calibration curve (curve 2) and its theoretical quantitative value (curve 3). It is apparent from the diagram that about two-thirds of the possible amount of nitrite ion is recovered. At least ten per cent of this is attributed to the alcoholic coupler solution as discussed earlier.

Excessive amounts of zinc tend to decrease the amount of nitrite ion formed. Liberties may be taken with the amount used in the range of 1/4 - 1 teaspoonful. Approximately equal portions should be used in successive trials. Attempts to employ a zinc column proved less successful than the stirring technique.

High temperatures may cause slight errors. It was found for example, that heat generated by the magnetic stirrer after prolonged use, was responsible for some deviation. This was remedied by placing two thin asbestos mats between the stirrer and flask.

Increased stirring rates were also possible when the mats were used. The decrease in magnetic attraction between the stirrer and stirring bar, due to greater distance, lessened the friction between the zinc and stirring bar and permitted faster rotation. A series of trials at lower temperatures showed no noticeable deviation from the results reported at room temperature.

The procedures recommended previously for nitrite ion and chlorine removal apply equally well to the present determination.

The following equation relating concentration to absorbance was derived from the nitrate ion calibration curve:

$$C_{NO_3^-} = 3.44\ A$$

where the symbols have the same connotation as those previously used.

It is the author's personal feeling that the modified nitrate ion determination just described is to be preferred over the one mentioned earlier. While some sensitivity is lost when partial reduction is used, the benefits gained by simplifying the procedure are a distinct advantage. For all practical purposes the precision of the modified method is comparable to the one involving complete nitrite ion recovery. This, plus the fact that the partial reduction method is easily adaptable to field work, tend to make it more applicable for general use.

CONCLUSION

Two procedures have been developed for the determination of nitrite and/or nitrate ions in water by utilizing the diazo-coupling reaction.

The first method, in which nitrite ion is recovered quantitatively, has greater sensitivity, while the second method, which depends on partial reduction, is simpler to perform. Both modifications were found to be suitable quantitative procedures.

For the first time, (4-aminophenyl)trimethylammonium ion has been used as an analytical reagent. It was found to be readily adaptable to the nitrite and nitrate ion determinations, and a valuable aid in the removal of nitrite ion. The latter use arising from the ease with which its diazonium cation can be isolated by ion exchange techniques.

A new method for the removal of residual chlorine, which is an interference to the nitrate ion determination, has been developed. Presumably, the reaction involved proceeds by direct chlorination or addition to a cation exchange resin.

The diazonium cation derived from (4-aminophenyl)trimethylammonium ion was found to be capable of coupling with various compounds. Its behavior in this respect was studied under varying pH conditions, and the colors of the azo dyes it formed with twenty-three biochemical reagents were noted. The coupling product produced by its reaction with phenol was further investigated, and found to obey Beer's Law in the concentration range up to 10 p.p.m.

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DIFFERENTIAL COLORIMETRIC DETERMINATION OF NITRITE AND NITRATE IONS IN WATER

by

FRED ZITOMER

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Nitrite and nitrate ions in the ranges of 0-1.5 and 0-2.0 p.p.m. respectively, are determined colorimetrically. Formation of a red dye by the diazo-coupling reaction serves as a means of identification. Nitrite ion is determined directly, while nitrate ion is first reduced and then determined by the same method. Interference encountered in the nitrate ion determination due to the presence of nitrite ion is eliminated by utilizing the latter to form a diazonium cation, which is then removed by cation exchange. Removal of chlorine and/or hypochlorous acid, which interfere, is accomplished through reaction with a nuclear sulfonic cation exchange resin, presumably by chlorination of the resin. Chloride ion does not interfere. The test is particularly applicable to the analysis of trace amounts of nitrite and/or nitrate ions in water.

A nitrate ion determination in the range of 0-5.0 p.p.m., utilizing partial reduction techniques, has the advantage of being easier to run, but is less sensitive than methods using total reduction.

A synthesis for the new analytical reagent, (4-aminophenyl)trimethylammonium ion, is described. Diazotization of this amine and subsequent coupling with N,N-dimethyl-1-naphthylamine produces the dye which is measured colorimetrically.

Some qualitative and quantitative aspects of (4-trimethyl-ammoniumphenyl)diazonium cation are discussed, with regard to its coupling ability in the presence of twenty-three biochemical reagents.